

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/105992/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Zhu, B, Caldwell, M and Song, Bing ORCID: <https://orcid.org/0000-0001-9356-2333> 2016. Development of stem cell-based therapies for Parkinson's disease. International Journal of Neuroscience 126 (11) , pp. 955-962.
10.3109/00207454.2016.1148034 file

Publishers page: <http://dx.doi.org/10.3109/00207454.2016.1148034>
<<http://dx.doi.org/10.3109/00207454.2016.1148034>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies.

See

<http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Development of stem cell-based therapies for Parkinson's disease

Bangfu Zhu¹ Maeve Caldwell² Bing Song^{1*}

¹ School of Dentistry, Cardiff Institute of Tissue Engineering and Repair (CITER), Cardiff University, Cardiff, UK

² School of Medicine, Trinity College Dublin, Ireland

*Corresponding author, Email: SongB3@cardiff.ac.uk

I. Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) in the brain with an unknown cause. Current pharmacological treatments for PD are only symptomatic and there is still no cure for this disease nowadays. In fact, transplantation of human fetal ventral midbrain cells into PD brains have provided a proof of concept that cell replacement therapy can be used for some PD patients, beneficial for improving their symptoms. However, the ethical and practical issues of human fetal tissue will inevitably limit its widespread clinical use. Therefore, it is essential to find alternative cell sources for the future cell transplantation for PD patients. With recent development in stem cell technology, here we review the different types of stem cells and their main properties currently explored, which could be developed as a possible cell therapy for PD treatment.

II. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, which affects about 1% of the population over the age of 60 [1]. One of the central pathological features of PD is the progressive loss of nigrostriatal dopamine (DA), which is accompanied by the presence of α -synuclein containing cytoplasmic inclusions known as Lewy bodies. The loss of DA neurons results in the characteristic motor symptoms of PD such as muscle rigidity, tremors and bradykinesia along with some of the cognitive deficits. Although the familial forms of the disorder are associated with some genetic defects, the aetiology of PD remains unknown in the vast majority of sporadic cases.

To date, there is no cure for this debilitating, neurological condition. Currently, the main clinical treatment for PD is dopamine replacement therapy using L-dihydroxyphenylalanine (L-DOPA) and/or dopamine receptor agonists. Pharmacotherapy can improve parkinsonian symptoms during the initial stage of PD, but the efficacy of pharmacotherapy is gradually lost and long-term treatment with L-DOPA consequently produces grave side effects, such as on-off fluctuations and drug-induced dyskinesias [2,3].

In addition, pharmacotherapy cannot delay the progression of the loss of DA neurons, and also cannot recover the lost DA neurons. As a result, there is a need for the development of regenerative therapies for PD.

Parkinson's disease (PD) is one of the candidate diseases suitable for cell transplantation therapy since successful clinical experiments have accumulated using human fetal tissue grafting for PD patients.

In pioneering work about three decades ago, it was shown that DA neuroblasts from the fetal ventral mesencephalon (VM) could survive long term in the adult brain and re-innervate the striatum of 6-hydroxydopamine (6-OHDA)-induced parkinsonian animal models, with a consequent improvement in most but not all of their behavioral responses [4-6].

Later the first clinical trial of cell transplantation therapy for PD patients was reported in 1987 using aborted human fetal VM tissue [7].

So far, several hundreds of PD patients have been treated as part of this type clinical trial. Some grafted PD patients have exhibited drastic improvements in their symptoms, but only

modest changes in others. These initial studies led on to bigger studies which then reported side effects in some patients in receipt of such grafts in the form of involuntary graft induced movements [8].

In addition, not enough fetal tissue is available to treat the large numbers of people with Parkinson's, and the use of fetuses also raises ethical concerns, which along with logistic and technical issues will significantly restrict extensive use of fetal tissue for the treatment of Parkinson's disease. Therefore, it is necessary to develop efficient methods to generate midbrain DA neurons from other possible sources such as stem cells instead of human VM tissue for the treatment of Parkinson's patients.

In this review, we highlight the recent advances in different DA induction protocols and characterization of various types of stem cells such as neural stem cells (NSCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), and will also discuss their prospects to replace lost midbrain DA neurons for the cell transplantation therapy for PD (Figure 1).

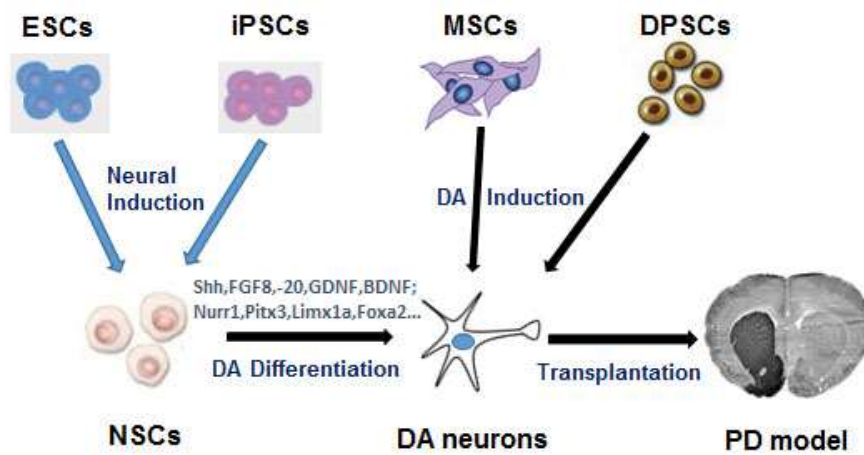


Figure 1. Schematic diagram of developing stem cells-derived Dopamine (DA) neurons for the treatment of Parkinson's disease (PD). ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; NSCs: neural stem/progenitor cells; MSCs: mesenchymal stem cells; DPSCs: dental pulp stem cells.

III. Stem cell sources for PD therapy

In order to move to clinical applications, a readily available and renewable source of cells with the potential to differentiate into fully functional DA neurons after transplantation is essential. If DA neurons can be generated from non-dopaminergic cell sources *in vitro* through the differentiation of stem cells, and subsequently transplanted into the brain with the

primary aim of providing appropriate, regulated levels of DA release in the striatum, then there is promise of constricting the disease process and recovering motor function in PD patients. Research has demonstrated that transplanted DA neurons generated from stem cells are capable of interconnecting with the host and these transplanted neurons can form neuronal circuitry, enabling directed DA release and the restoration of normal movements.

1. Neural stem cells(NSCs)

Neural stem cells (NSCs) or progenitor cells (NPCs) can be derived from embryonic stem cell sources, and they have also been shown to exist in the adult brain [9]. Transplantation of NSCs in the brain attenuates physical or functional deficits associated with injury or disease in the CNS via cell replacement, the release of specific neurotransmitters, and the production of neurotrophic factors that protect injured neurons and promote neuronal growth.

Adult neural stem cells are mainly in the hippocampus and subventricular zone (SVZ) near the lateral. Madhavan and colleagues reported stimulation of endogenous cells after transplantation and hypothesized that there exists a synergism between the actions of endogenous and grafted NPCs after transplantation that would lead to neuroprotection in a 6-OHDA rat model of PD [10]. However, controversy regarding neurogenesis in the SVZ in PD models persists [11].

Neural stem cells (NSCs) obtained from human fetal tissue were the pioneered cell type studied for PD therapy following the isolation and propagation of NSCs [12]. Since then, researchers have employed varying methods to generate DA neurons from NSCs, including treating the cells with cytokines and neurotrophic factors such as GDNF *in vitro* [13]. It has also been demonstrated the transplanted NPCs derived DA cells illustrated high survival rates which improved the symptoms of PD [14], and human fetal neural stem cells survive long term in the midbrain of dopamine-depleted monkeys after GDNF overexpression and project neurites toward an appropriate target [15]. Consistently, intrastriatal GDNF gene transfer by inducible lentivirus vectors can protect dopaminergic neurons in a rat model of parkinsonism and intrastriatal transplantation of adult human neural crest-derived stem cells improves functional recovery in parkinsonian rats [16, 17].

The important role of Bcl-x_L has been revealed in the generation of DA neurons from NSCs. Transcription factors associated with the production of DA neurons such as *Nurr1*, *Pitx3*, *Lmx1b*, and *DAT* are induced by Bcl-x_L establishing a high yield of DA neurons characteristic of the lost cells in the SNpc [18]. We have demonstrated when *Pitx3* was overexpressed in NSCs, there was an increase in the differential potential of the generated DA neurons and a substantial improvement in motor function was observed following the transplantation of the grafts containing VM and NSCs overexpressed with *Pitx3* in a 6-OHDA animal model [19]. Whereas co-expression of *Nurr1* and *Brn4* promoted NSCs to differentiate into dopaminergic neurons *in vivo*, increased the level of DA neurotransmitter in the striatum, resulting in behavioral improvement of PD rats. *Brn4* and tyrosine hydroxylase (TH) synergistically promote the differentiation of neural stem cells into dopaminergic neurons and increase cell survival and DA release of dopaminergic neurons [20,21]. Recently, it has been found that induced neural stem cells (iNSCs) that over-expressed *Lmx1a* (iNSC-*Lmx1a*) gave rise to an increased yield of dopaminergic neurons and secreted a higher level of dopamine *in vitro* and mice that received iNSC-*Lmx1a* vs. iNSC-GFP exhibited better recovery [22].

However, major hurdles in relation to the use of NSCs for the generation of DA neurons need to be overcome before they can be clinically applied for the treatment of PD. It is essential that transplants of NSC/progenitor cells justify their capability to improve the refined motor problems of PD, and long-term culturing of NSC/progenitors and their stable differentiation potential can be guaranteed [13].

Therefore, it has been proposed that the governed differentiation of induced pluripotent stem cells (iPSs) or ESC-human derived cells may be better employed for the future generation of DA neuronal cell types, although one advantage of NSCs is that they have yet to be shown to form the same type of tumours after transplantation.

2. Embryonic stem cells (ESCs)

Embryonic stem cells (ESCs) have the ability to develop into any cell type of the body. Research has revealed that ESCs may generate DA neurons at a specific developmental stage; that is prior to or during the cells' fate to a neural phenotype, therefore influencing the application of sonic hedgehog (Shh) and fibroblast growth factor 8 (FGF8) at the height of the production of neural Nestin⁺ and Sox1⁺ progenitors. At the ESC differentiation stage, factors such as Shh and FGF8 can be induced to provide a ~30% TH⁺ neuronal population observed in culture [23]. Another study has shown that differentiation of human ES cells into VM dopaminergic neurons requires a high activity form of Shh, FGF8a and specific regionalization by retinoic acid (RA) [24].

In addition, when NPCs derived from ESCs are exposed *in vitro* to both FGF8 and Shh, they are capable of differentiating into Lmx1a-DA neurons [25]. Another study illustrated the usefulness of FGF20 and FGF2 involved in the differentiation of DA neurons from human ESCs. These findings will aid the efficient generation of DA neurons from ESC required for cell replacement therapies for PD [26]. Hong and associates have established that overexpression of each factors, Nurr1, Pitx3, and Lmx1a robustly promoted the dopaminergic differentiation of ESC-NP cells exposed to SHH and FGF8 and demonstrated that key midbrain DA (mDA) regulators (Nurr1, Pitx3, and Lmx1a) play overlapping as well as distinct roles during neurogenesis and neurotransmitter phenotype determination of mDA neurons. This further proved the link of generation of DA neurons from ESCs with the overexpression of genes and transcription factors, such as *Nurr1*, *Pitx3* and *Lmx1a* [27,28]. Human ESCs also display electrophysiological features of DA neurons and most importantly are capable of DA release *in vitro* whilst demonstrating gene expression. Several studies have illustrated methods to enhance the generation of DA neurons from human ESCs [28,29]. These findings highlight the necessity for the identification of differentiation and survival mechanisms and the eradication of factors responsible for excessive proliferation, required for protocols for cell replacement therapies today. Scientists have also illustrated that a high percentage of ESCs do not undergo differentiation into the desired cell type when those differentiation protocols are applied [8,29].

Using protocols entirely based on extrinsic patterning cues that mimic fetal midbrain development, it is now possible to generate DA neurons with a midbrain phenotype from human embryonic stem cells that survive transplantation and that can restore motor deficits in animal models of PD [30-33]. More remarkably, Grealish and colleagues have shown long-term survival and functionality using clinically relevant MRI and PET imaging techniques and demonstrate efficacy in restoration of motor function with a potency comparable to that seen with human fetal dopamine neurons. Furthermore, hESC-derived dopamine neurons can project sufficiently long distances for use in humans, fully regenerate midbrain-to-forebrain projections, and innervate correct target structures. This provides strong preclinical support for clinical translation of hESC-derived dopamine neurons using approaches similar to those established with fetal cells for the treatment of Parkinson's disease [34].

Among the different stem cell sources available, human ES cells have advanced a great deal. However, a number of crucial issues still need to be addressed in preclinical studies before these cells can be considered for clinical use: it is important to verify that their functional efficacy is robust and stable over significantly long time periods; The incomplete differentiation of all ESCs is a major challenge for the development of an effective PD therapy and the risk associated with tumourigenesis may hinder the applications of ESCs [8].

3. Induced pluripotent stem cells (iPSCs)

Induced pluripotent stem cells (iPSCs) are an advantageous cell replacement source for PD as these autologous stem cells can be derived from the patient eliminating the risk of immune rejection and the requirement of immunosuppressive therapy. Unlike human ESCs, iPS cells are not hindered by ethical issues [35].

Research studies carried out using animal models displayed promising results for iPS cells in the generation of DA neurons. An experimental study was carried out using mouse fibroblasts to produce iPS cells. It was observed that the generated iPS cells could be differentiated into DA neurons of the midbrain which subsequently diminished motor asymmetry in 6-OHDA rodent models induced with lesions [36].

Shh, FGF8, FGF2 and retinoic acid (RA) together successfully pre-differentiated iPS cells into functional midbrain DA neurons. This led to the integration of the DA neurons into the host striatum of rodent models induced with PD, resulting in an enhancement of **behavior** [24,37]. However, like ESC grafts, the development of neural outgrowths **was** observed as a result of the transplanted cells [38].

It has also revealed that DA neurons can be generated from human iPS cells, as well as from mouse iPS cells. Human fibroblasts have been used to generate iPS cells which subsequently resulted in the generation of TH+ neurons [39]. The iPS cell-derived NSCs were also found to survive and integrate into the brain and differentiated into neurons, including DA neurons *in vivo* and improved functional recovery of PD rats up to 16 weeks after transplantation. [40].

Work by Hallett and colleagues showed pre-clinical test of transplantation of autologous iPSC-derived dopamine neurons in a cynomolgus monkey model of Parkinson's disease provides proof of principle for long-term innervation and functional benefit without a requirement for immunosuppression [41].

It has also been shown that human iPSC-derived DA progenitor cells can be efficiently isolated by cell sorting using a floor plate marker, CORIN. After induction, the sorted CORIN(+) cells expressed the midbrain DA progenitor markers, FOXA2 and LMX1A. When transplanted into 6-OHDA-lesioned rats, the CORIN(+) cells survived and differentiated into midbrain DA neurons *in vivo*, resulting in significant improvement of the motor behavior, without tumor formation [42].

As we know, a frequent cause of familial PD arises from mutations in LRRK2. Dopamine neurons derived from mutated LRRK2 iPSCs showed increased expression of α -synuclein, suggesting a connection between these two risk genes in a pathogenic pathway, as had been previously hypothesized [43]. A subsequent study also indicated phenotypic differences between LRRK2 mutant cells and control cells [44]. PD iPSCs from Parkin patients also showed evidence of increased oxidative stress and enhanced activity of the related NRF2 pathway [45].

In the meantime, PD iPSCs differentiated into dopamine neurons could be transplanted into the adult rodent striatum, and developed axons projecting into the striatum. 6-OHDA-lesioned rats transplanted with the neurons showed reduced amphetamine-induced rotational asymmetry [46]. Similarly, transplantation of human protein-based iPSCs into rats with striatal lesions could rescue motor deficits [47].

In this context, the ability to reprogram somatic cells to pluripotency represents a promising new tool in disease modelling and drug discovery for neurodegenerative diseases, such as PD as iPSCs contain the entire genome of the patient and thus have potential utility in studying early disease initiating events and developmental progression of the disease, as well as the disease pathology [48].

However, a number of issues need to be addressed as this cell type derived from PD sufferers are associated with mutations, polymorphisms or epigenetic marks, making them exposed to

the occurrence of PD-like characteristics [38]. Additionally some reprogramming factors and/or viral vectors integrated into the genome increases the risk of mutations and tumorigenesis [49].

In brief, human induced pluripotent stem cells (iPSCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy for Parkinson's disease. **One of the studies has highlighted their ability to generate 20-40% of neurons derived from iPS cells into a DA phenotype [13].** Nevertheless, iPSC-derived donor cells may contain tumorigenic or inappropriate cells.

4. Mesenchymal stem cells (MSCs)

Mesenchymal stem cells (MSCs) possess several attractive properties for use as a novel therapeutic for neurodegenerative disorders, including PD as they can be easily extracted, cultured and expanded. Many studies in PD animal models have verified that bone marrow-derived MSCs (BMSCs) have the capacity to protect and regenerate damaged DA neurons. [50].

It was first demonstrated that behavioral recovery after BMSCs transplantation in MPTP-induced mouse model of PD [51]. Later, increased viability and migration of transplanted BMSCs were observed after 6-OHDA-induced loss of DA neurons [52]. In addition, BMSCs grafted into the striatum [53] intranasally [54] or intravenously [55] delivered BMSCs were also evident to exert neuroprotective effects against nigrostriatal degeneration and to improve motor function in 6-OHDA lesioned rats. Human BMSCs also have a protective effect on the progressive loss of DA neurons in vitro and in vivo in rat [56].

When neuralized BMSCs were transplanted in a 6-OHDA mouse model of PD, most of the transplanted cells survived in striatum, expressed TH and behavioral recovery was observed [57]. In addition, micrografted bone marrow derived neuroprogenitor-like cells were shown to induce rejuvenation of host DA neurons in 6-OHDA partially lesioned rat brain [58]. MSCs isolated from other sources, such as adipose tissue and umbilical cord, have also shown beneficial effects in PD models as well. Furthermore, the naive and neurally-induced adipose derived MSCs have neuroprotective effects against 6-OHDA-induced DA neuron death through secretion of neurotrophic factors [59]. Interestingly, MSCs isolated from umbilical cord exhibit neuroprotective and neuroregenerative effects in 6-OHDA [60,61] and in vitro-generated first trimester placental MSCs-derived neural progenitors are capable of terminal differentiation in vivo and can attenuate motor defects associated with PD [62]. MSCs are unique compared to other stem cells in that they could theoretically be utilized for personalized medicine in which MSCs for brain engraftment would be collected from the individual to receive grafted cells in order to avoid immune responses and graft rejection [50].

5. Other stem cells

The Oral and/or dental stem cells, including dental pulp stem cells (DPSCs) and oral mucosa stem cells, have recently been explored for the use beyond dentistry as they have attractive virtues of easy access and autografting possibility. Some of these oral/dental stem cells have shown the abilities of neural differentiation and neuroprotection, hence have great potential for treating neurodegenerative diseases [63].

For example, Nesti and colleagues have found the co-culture with DPSCs significantly attenuated MPP+ or rotenone-induced toxicity in primary cultures of mesencephalic neurons, presumably due to soluble factors such as BDNF and NGF released by DPSCs [64]. Later, it has been demonstrated that some soluble factors involved in the development of DA neurons induced a DA phenotype in human oral mucosa stem cells (**hOMSCs**) in vitro that significantly improved the motor function of hemiparkinsonian rats. **Immunofluorescence analysis of specific DA marker of naive and differentiated hOMSCs showed that 35% and 82% were positive for TH respectively [65].**

More recently, a study showed engrafted DAergic neuron-like SHEDs (stem cells derived from human exfoliated deciduous teeth) survived in the striatum of Parkinsonian rats, improved the DA level more efficiently than engrafted undifferentiated SHEDs, and also promoted the recovery from neurological deficits, which suggests that stem cells derived from dental pulp have therapeutic benefits for PD [66].

Studies have also illustrated that the bone marrow, skin, muscles and adipose tissue can generate cells staining positive for neuronal markers. However, this group of cells is not an advantageous source for the generation of DA neurons for PD [67]. More research is needed for the enhancement of adult stem cell isolation, culture procedures and differentiation protocols if there is any hope for them to be used for PD treatment.

IV. Perspectives

As discussed above many types of stem cells, such as ESCs, iPSCs and NSCs, can be induced into dopaminergic or dopaminergic-like neurons for the treatment of Parkinsonian models. Understandably each type of stem cells has pros and cons in terms of their efficacy, safety and availability. At this point, it looks still an open race which cell type will be the best solution for the future cell replacement treatment of PD although some people may believe that ESCs and iPSCs are the front runners at the moment.

So far human fetal VM cells are the only one clinically proved functional for some Parkinson's patients, thus can serve as a gold standard for stem cell-derived DA transplantation for the moment at least.

Besides the important focus on cell sources and differentiation protocols, the optimal transplantation strategies should also need to be further studied before any clinical applications of these stem cells.

One of the issues is how to find a better and more reliable delivery system for stem cell transplantation in future. With the advances in biomaterials and nanomaterials, ideal scaffolds could be developed soon for clinical stem cell replacement, particularly when it is necessary to combine with growth factors and/or gene therapy. These materials should be biocompatible as well as biodegradable so that they can accommodate implanted cells with physical support/protection in a 3D environment, and also help the cells access to nutrients and integrate into neural network in vivo.

Another concern is the identity and/or characteristics of the stem cells and the derived progeny, i.e. do they proliferate in a controlled manner and not differentiate into non-dopaminergic or even tumor cells?

Other issues such as transplantation locations and timing also need to be further explored before clinical use of stem cells-derived dopaminergic neurons for the transplantation into Parkinson's patients.

All in all, although cell replacement therapy for PD is in sight, maybe we still need to wait until the time is right [8].

V. Acknowledgements

This work was supported by the European Research Council StG Grant 243261.

VI. References

1. Lang AE, Lozano AM. Parkinson's disease. First of two parts. N Engl J Med. 1998; 339: 1030–1043.
2. Lang AE, Lozano AM. Parkinson's disease. Second of two parts. N Engl J Med 1998; 339: 1044–1053.

3. Meissner WG, Frasier M, Gasser T. Priorities in Parkinson's disease research. *Nat Rev Drug Discov.* 2011; 10 (5):377–393.
4. Dunnett SB, Björklund A, Schmidt RH, Stenevi U, Iversen SD. Intracerebral grafting of neuronal cell suspensions. V. Behavioural recovery in rats with bilateral 6-OHDA lesions following implantation of nigral cell suspensions. *Acta Physiol Scand Suppl.* 1983; 522:39–47.
5. Freund TF, Bolam JP, Björklund A, et al. Efferent synaptic connections of grafted dopaminergic neurons reinnervating the host neostriatum: a tyrosine hydroxylase immunocytochemical study. *J Neurosci.* 1985; 5(3):603–616.
6. Annett LE, Torres EM, Clarke DJ, et al. Survival of nigral grafts within the striatum of marmosets with 6-OHDA lesions depends critically on donor embryo age. *Cell Transplant.* 1997;6(6):557–569.
7. Brundin P, Strecker RE, Lindvall O, Isacson O, Nilsson OG, Barbin G, Prochiantz A, Forni C, Nieoullon A, Widner H, Gage FH, Björklund A. Intracerebral grafting of dopamine neurons. Experimental basis for clinical trials in patients with Parkinson's disease. *Ann. N. Y. Acad. Sci.* 1987; 495: 473–496.
8. Barker RA. Developing Stem Cell Therapies for Parkinson's Disease: Waiting Until the Time Is Right. *Cell Stem Cell.* 2014; 15: 540-542.
9. Horiguchi S, Takahashi J, Kishi Y. Neural precursor cells derived from human embryonic brain retain regional specificity. *J Neurosci Res.* 2004; 75(6):817–824.
10. Madhavan L, Daley BF, Paumier KL, Collier TJ. Transplantation of subventricular zone neural precursors induces an endogenous precursor cell response in a rat model of Parkinson's disease. *J Comp Neurol.* 2009; 515:102–115.
11. Mochizuki H, Choong CJ, Yasuda T. The promises of stem cells: stem cell therapy for movement disorders. *Parkinsonism and Related Disorders.* 2014; 20S1:S128–S131
12. Reynolds BA, Tetzlaff W, Weiss S. A multipotent EGF-responsive striatal embryonic progenitor cell produced neurons and astrocytes. *J. Neurosci.* 1992; 12: 4565-4574
13. Fricker-Gates RA. And Gates MA. Stem cell-derived dopamine neurons for brain repair in Parkinson's disease. *Regen. Med.* 2010; 5(2): 267-278.
14. Redmond DE Jr, Bjugstad KB, Teng YD, Ourednik V, Oudernik J, Wakeman DR, Parsons XH, Gonzalez R, Blanchard BC, Kim SU, Gu Z, Lipton SA, Markakis EA, Roth RH, Elsworth JD, Slader JR Jr, Sidman RL. And Snyder EM. Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. *Proc. Natl Acad. Sci.* 2007; 104: 12175-12180.
15. Wakeman DR, Redmond DE Jr, Dodiya HB, Sladek JR Jr, Leranthe C, Teng YD, Samulski RJ, Snyder EY. Human neural stem cells survive long term in the midbrain of dopamine-depleted monkeys after GDNF overexpression and project neurites toward an appropriate target. *Stem Cells Transl Med.* 2014; 3(6):692-701.
16. Chen S, Yang C, Hao F, Li C, Lu T, Zhao L, Duan WM, Intrastriatal GDNF gene transfer by inducible lentivirus vectors protects dopaminergic neurons in a rat model of parkinsonism. *Experimental Neurology.* 2014; 261:87–96
17. Müller J, Ossig C, Greiner JF, Hauser S, Fauser M, Widera D, Kaltschmidt C, Storch A, Kaltschmidt B. Intrastriatal transplantation of adult human neural crest-derived stem cells improves functional outcome in parkinsonian rats. *Stem Cells Transl Med.* 2015; 4(1):31-43.
18. Courtois ET, Castillo CG, Seiz EG, Ramos M, Bueno C, Liste I and Martinez-Serrano A. In Vitro and In vivo enhanced generation of Human A9 Dopamine Neurons from Neural Stem Cells by BCL-XL. 2010; The American Society for Biochemistry and Molecular Biology, Inc.
19. O'Keefe FE, Scott SA, Tyers P. O'Keefe GW, Dalley JW, Zufferey R. and Caldwell MA. Induction of A9 dopaminergic neurons from neural stem cells improves motor function in an animal model of Parkinson's disease. *Brain.* 2008; 131: 630-641

20. Tan X, Zhang L, Qin J, Tian M, Zhu H, Dong C, Zhao H, Jin G. Transplantation of neural stem cells co-transfected with Nurr1 and Brn4 for treatment of Parkinsonian rats. *Int J Dev Neurosci*. 2013; 31(1):82-7.
21. Tan X, Zhang L, Zhu H, Qin J, Tian M, Dong C, Li H, Jin G. Brn4 and TH synergistically promote the differentiation of neural stem cells into dopaminergic neurons. *Neurosci Lett*. 2014; 571:23-8.
22. Wu J, Sheng C, Liu Z, Jia W, Wang B, Li M, Fu L, Ren Z, An J, Sang L, Song G, Wu Y, Xu Y, Wang S, Chen Z, Zhou Q, Zhang YA. Lmx1a enhances the effect of iNSCs in a PD model. *Stem Cell Res*. 2015; 14(1):1-9.
23. Parmar M. and Li M. Early specification of dopaminergic phenotype during ES cell differentiation. *BMC Dev Biol*. 2007; 7(1):86
24. Cooper O, Hargus G, Deleidi M, Blak A, Osborn T, Marlow E, Lee K, Levy A, Perez-Torres E, Yow A, Isacson O. Differentiation of human ES and Parkinson's disease iPS cells into ventral midbrain dopaminergic neurons requires a high activity form of SHH, FGF8a and specific regionalization by retinoic acid. *Mol Cell Neurosci*. 2010; 45(3):258-66.
25. Baizabal JM and Covarrubias L. The embryonic midbrain directs neuronal specification of embryonic stem cells at early stages of differentiation. *Dev. Biol*. 2008; 325(1)
26. Shimada H, Yoshimura N, Tsuji A and Kunisada T Differentiation of dopaminergic neurons from human embryonic stem cells: Modulation of differentiation by FGF-20. *Journal of Bioscience and Bioengineering*. 2009; 107(4): 447-454
27. Cai J, Donaldson A, Yang M, German MS, Enikolopov G, Iacovitti L. The Role of Lmx1a in the Differentiation of Human Embryonic Stem Cells into Midbrain Dopamine Neurons in Culture and After Transplantation into a Parkinson's Disease Model. *Stem Cells*. 2009; 27:220-229
28. Hong S, Chung S, Leung K, Hwang I, Moon J, Kim KS. Functional roles of Nurr1, Pitx3, and Lmx1a in neurogenesis and phenotype specification of dopamine neurons during in vitro differentiation of embryonic stem cells. *Stem Cells Dev*. 2014; 23(5):477-87.
29. Kriks S And Studer L Protocols for Generating ES Cell-Derived Dopamine Neurons. *Advances in experimental medicine and biology*. 2008; 651:101-111.
30. Chung S, Moon JI, Leung A, Aldrich D, Lukianov S, Kitayama Y, Park S, Li Y, Bolshakov VY, Lamonerie T, Kim KS. ES cell-derived renewable and functional midbrain dopaminergic progenitors. *Proc Natl Acad Sci U S A*. 2011;108 (23):9703-8.
31. Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z, Carrillo-Reid L, Auyeung G, Antonacci C, Buch A, Yang L, Beal MF, Surmeier DJ, Kordower JH, Tabar V, Studer L. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature*. 2011; 480 (7378):547-51.
32. Kirkeby A., Grealish S., Wolf D.A., Nelander J., Wood J., Lundblad M., Lindvall O., Parmar M. Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. *Cell Rep*. 2012; 1(6):703-14.
33. Wakeman DR, Weiss S, Sladek JR, Elsworth JD, Bauereis B, Leranthe C, Hurley PJ, Roth RH, Redmond DE. Survival and integration of neurons derived from human embryonic stem cells in MPTP-lesioned primates. *Cell Transplant*. 2014; 23(8):981-94.
34. Grealish S, Diguett E, Kirkeby A, Mattsson B, Heuer A, Bramoulle Y, Van Camp N, Perrier AL, Hantraye P, Björklund A and Parmar M. Human ESC-Derived Dopamine Neurons Show Similar Preclinical Efficacy and Potency to Fetal Neurons when Grafted in a Rat Model of Parkinson's Disease. *Cell Stem Cell*. 2014; 15(5): 653–665.
35. Lindvall O. And Kokaia Z. Prospects of stem cell therapy for replacing dopamine neurons in Parkinson's disease. *Trends in Pharmacological Sciences*. 2009; 30:5.
36. Martinez-Serrano A. and Liste I. Recent progress and challenges for the use of stem cell derivatives in neuron replacement therapy for Parkinson's disease *Future Neurol*. 2010; 5(2): 161-165.

37. Wernig M, Zhao JP, Pruszak J. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc. Natl Acad. Sci. USA*. 2008; 105: 5856-5861.
38. Arenas E. Towards stem cell replacement therapies for Parkinson's disease. *Biochemical and Biophysical Research Communications*. 2010; 396: 152-156.
39. Huangfu D, Osafune K., Maehr R. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat. Biotechnol.* 2008; 26: 1269-1275.
40. Han F, Wang W, Chen B, Chen C, Li S, Guo W, Li G. Human induced pluripotent stem cell-derived neurons improve motor asymmetry in a 6-hydroxydopamine-induced rat model of Parkinson's disease. *Cytotherapy*. 2015; 17(5): 665-679.
41. Hallett PJ, Deleidi M, Astradsson A, Smith GA, Cooper O, Osborn TM, Sundberg M, Moore MA, Perez-Torres E, Brownell AL, Schumacher JM, Spealman RD and Isacson O. Successful Function of Autologous iPSC-Derived Dopamine Neurons following Transplantation in a Non-Human Primate Model of Parkinson's Disease. *Cell Stem Cell*, 2015; 16(3): 269-274.
42. Doi D, Samata B, Katsukawa M, Kikuchi T, Morizane A, Ono Y, Sekiguchi K, Nakagawa M, Parmar M, Takahashi J. Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. *Stem Cell Rep*. 2014; 2:337-350.
43. Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, Kee K, Schule B, Dolmetsch RE, Langston W. LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. *Cell Stem Cell*. 2011; 8: 267-280.
44. Cooper O, Seo H, Andrabi S, Guardia-Laguarta C, Graziotto J, Sundberg M, McLean JR, Carrillo-Reid L, Xie Z, Osborn T. Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. *Sci. Transl. Med.* 2012; 4: 141ra190.
45. Imaizumi Y, Okada Y, Akamatsu W, Koike M, Kuzumaki N, Hayakawa H, Nihira T, Kobayashi T, Ohyama M, Sato S. Mitochondrial dysfunction associated with increased oxidative stress and alpha-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. *Mol. Brain*. 2012; 5:35.
46. Hargus G, Cooper O, Deleidi M, Levy A, Lee K, Marlow E, Yow A, Soldner F, Hockemeyer D, Hallett PJ. Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proc. Natl. Acad. Sci. USA*. 2010; 107: 15921-15926.
47. Rhee YH, Ko JY, Chang MY, Yi SH, Kim D, Kim CH, Shim JW, Jo AY, Kim BW, Lee H. Protein-based human iPS cells efficiently generate functional dopamine neurons and can treat a rat model of Parkinson disease. *J. Clin. Invest.* 2011; 121: 2326-2335.
48. Ross CA and Akimov SS. Human-induced pluripotent stem cells: potential for neurodegenerative diseases. *Human Molecular Genetics*. 2014; 23(R1): R17-R26
49. Chun C, LingJun R, LinZhao C And Lei X. Generation and application of human iPS cells. *Chinese Science Bulletin*. 2009; 54 (1): 9-13.
50. Glavaski-Joksimovic A and Bohn MC. Mesenchymal stem cells and neuroregeneration in Parkinson's disease. *Experimental Neurology*. 2013; 247:25-38
51. Li Y, Chen J, Wang L, Zhang L, Lu M, Chopp M. Intracerebral transplantation of bonemarrowstromal cells in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinemouse model of Parkinson's disease. *Neurosci. Lett*. 2001; 316: 67-70.
52. Hellmann MA, Panet H, Barhum Y, Melamed E, Offen D. Increased survival and migration of engrafted mesenchymal bone marrow stem cells in 6-hydroxydopamine-lesioned rodents. *Neurosci. Lett*. 2006; 395: 124-128.
53. Blandini F, Cova L, Armentero MT, Zennaro E, Levandis G, Bossolasco P, Calzarossa C, Mellone M, Giuseppe B, Delilieri GL, Polli E, Nappi G, Silani V. Transplantation of

- undifferentiated human mesenchymal stem cells protects against 6-hydroxydopamine neurotoxicity in the rat. *Cell Transplant.* 2010; 19: 203–217.
54. Danielyan L, Schafer R, von Ameln-Mayerhofer A, Bernhard F, Verleysdonk S, Buadze M, Lourhmati A, Klopfer T, Schaumann F, Schmid B, Koehle C, Proksch B, Weissert R, Schwab M, Gleiter CH, Frey II WH. Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of Parkinson disease. *Rejuvenation Res.* 2011; 14:3–16.
 55. Suzuki S, Kawamata J, Iwahara N, Matsumura A, Hisahara S, Matsushita T, Sasaki M, Honmou O, Shimohama S. Intravenous mesenchymal stem cell administration exhibits therapeutic effects against 6-hydroxydopamine-induced dopaminergic neurodegeneration and glial activation in rats. *Neuroscience Letters.* 2015; 584: 276–281.
 56. Park HJ, Lee PH, Bang OY, Lee G, Ahn YH. Mesenchymal stem cells therapy exerts neuroprotection in a progressive animal model of Parkinson's disease. *J. Neurochem.* 2008; 107: 141–151.
 57. Offen D, Barhum Y, Levy YS, Burshtein A, Panet H, Cherlow T, Melamed E. Intrastriatal transplantation of mouse bone marrow-derived stem cells improves motor behavior in a mouse model of Parkinson's disease. *J. Neural Transm.* 2007; Suppl.133–143.
 58. Glavaski-Joksimovic A, Virag T, Chang QA, West NC, Mangatu TA, McGrogan MP, Dugich-Djordjevic M, Bohn MC. Reversal of dopaminergic degeneration in a parkinsonian rat following micrografting of human bone marrow-derived neural progenitors. *Cell Transplant.* 2009; 18:801–814.
 59. McCoy MK, Martinez TN, Ruhn KA, Wrage PC, Keefer EW, Botterman BR, Tansey KE, Tansey MG. Autologous transplants of adipose-derived adult stromal (ADAS) cells afford dopaminergic neuroprotection in a model of Parkinson's disease. *Exp. Neurol.* 2008; 210: 14–29.
 60. Mathieu P, Roca V, Gamba C, Del Pozo A, Pitossi F. Neuroprotective effects of human umbilical cord mesenchymal stromal cells in an immunocompetent animal model of Parkinson's disease. *J. Neuroimmunol.* 2012; 246: 43–50.
 61. Xiong N, Zhang Z, Huang J, Chen C, Zhang Z, Jia M, Xiong J, Liu X, Sun S, Lin Z and Wang T. VEGF-expressing human umbilical cord mesenchymal stem cells, an improved therapy strategy for Parkinson's disease. *Gene Therapy.* 2011; 18: 394–402.
 62. Park S, Kima E, Kohb SE, Maeng S, Leed W, Lima J, Shime I, Lee YJ. Dopaminergic differentiation of neural progenitors derived from placental mesenchymal stem cells in the brains of Parkinson's disease model rats and alleviation of asymmetric rotational behaviour. *Brain Research.* 2012; 1466: 158–166.
 63. Jiang W, Ni L, Sloan A, Song B. *Tissue Engineering and Regenerative Medicine, From and Beyond the Dentistry.* Dentistry. 2015; 5:306.
 64. Nesti C, Pardini C, Barachini S, D'Alessandro D, Siciliano G, Murri L, Petrini M, Vaglini F. Human dental pulp stem cells protect mouse dopaminergic neurons against MPP+ or rotenone. *Brain Res.* 2011; 1367:94-102.
 65. Ganz J, Arie I, Buch S, Zur TB, Barhum Y, Pour S, Araidy S, Pitaru S, Offen D. Dopaminergic-like neurons derived from oral mucosa stem cells by developmental cues improve symptoms in the hemi-parkinsonian rat model. *PLoS One.* 2014; 9(6):e100445.
 66. Fujii H, Matsubara K, Sakai K, Ito M, Ohno K, Ueda M, Yamamoto A. Dopaminergic differentiation of stem cells from human deciduous teeth and their therapeutic benefits for Parkinsonian rats. *Brain Res.* 2015; 1613:59-72.
 67. Toulouse A. and Sullivan AM. Progress in Parkinson's disease- Where do we stand? *Progress in Neurobiology.* 2008; 85:376-392.